

Amplified Interactive Toxicity of Chemicals at Nontoxic Levels: Mechanistic Considerations and Implications to Public Health

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It is widely recognized that exposure to combinations or mixtures of chemicals may result in highly exaggerated toxicity even though the individual chemicals might not be toxic. Assessment of risk from exposure to combinations of chemicals requires the knowledge of the underlying mechanism(s). Dietary exposure to a nontoxic dose of chlordecone (CD; 10 ppm, 15 days) results in a 67-fold increase in lethality of an ordinarily inconsequential dose of CCl_4 (100 $\mu\text{l/kg}$, ip). Toxicity of closely related CHCl_3 and BrCCl_3 is also enhanced. Phenobarbital (PB, 225 ppm, 15 days) and mirex (10 ppm, 15 days) do not share the propensity of CD in this regard. Exposure to PB + CCl_4 results in enhanced liver injury similar to that observed with CD, but the animals recover and survive in contrast to the greatly amplified lethality of CD + CCl_4 . Investigations have revealed that neither enhanced bioactivation of CCl_4 nor increased lipid peroxidation offers a satisfactory explanation of these findings. Additional studies indicate that exposure to a low dose of CCl_4 (100 $\mu\text{l/kg}$, ip) results in limited injury, which is accompanied by a biphasic response of hepatocellular regeneration (6 and 36 hr) and tissue repair, which enables the animals to recover from injury. Exposure to CD + CCl_4 results in suppressed tissue repair owing to an energy deficit in hepatocytes as a consequence of excessive intracellular influx of Ca^{2+} leading initially to a precipitous decline in glycogen and ultimately to hypoglycemia. Supplementation of cellular energy results in restoration of the tissue repair and complete recovery from the toxicity of CD + CCl_4 combination. In contrast, only the early-phase hepatic tissue repair (6 hr) is affected in PB + CCl_4 treatment, but this is adequately compensated for by a greater stimulation of tissue repair at 24 and 48 hr resulting in recovery from liver injury and animal survival. A wide variety of additional experimental evidence confirms the central role of stimulated tissue repair as a decisive determinant of the final outcome of liver injury inflicted by CCl_4 . For instance, a 35-fold greater CCl_4 sensitivity of gerbils compared to rats is correlated with the very sluggish tissue repair in gerbils. These findings are consistent with a two-stage model of toxicity, where tissue injury is inflicted by the well described "mechanisms of toxicity," but the outcome of this injury is determined by whether or not sustainable tissue repair response accompanies this injury. These findings impact significantly on our ability to predict the ultimate outcome of toxic injury and form a firm basis for additional mechanism-driven investigations into the endogenous tissue repair response evoked by tissue injury. These concepts will enhance our ability to fine-tune the tools of risk assessment such as animal-to-man extrapolation and prediction of ultimate outcome of toxic injury. —*Environ Health Perspect* 102(Suppl 9):139–149 (1994)

Key words: chlordecone (Kepone), carbon tetrachloride, chloroform, bromotrichloromethane, hormesis, tissue repair, hepatic regeneration, two-stage model of toxicity, amplified toxicity, mechanism, risk assessment

Introduction

From a perspective of public health, a major toxicological issue is the possibility of unusual toxicity due to interaction of two or more toxic chemicals at individually harmless levels upon environmental or occupational exposures. While some laboratory models exist for such interactions

involving two chemicals, progress in this area has suffered for want of models where the two interactants are individually nontoxic. Toxicities resulting from exposure to more than two chemicals at individually nontoxic doses are of greater interest since this exposure scenario is most common. One such model is available, where prior exposure to nontoxic levels of the pesticide Kepone (Chlordecone) results in a 67-fold amplification of CCl_4 lethality in rats (Table 1). The mechanism of this remarkable interactive toxicity is of interest in the assessment of risk from exposure to combinations of chemicals.

Amplified Toxicity of CCl_4 by Chlordecone

Prior exposure to a nontoxic level of chlordecone (10 ppm in diet for 15 days) results in a marked amplification of CCl_4 hepatotoxicity (1–3) and lethality (3–5). Neither the close structural analogs of chlordecone, mirex and photomirex, nor

phenobarbital (Figure 1), exhibit this property (2,3). Plaa and associates (6,7) have demonstrated the capacity of chlordecone to potentiate CHCl_3 hepatotoxicity in mice. These observations have been extended to demonstrate that, in addition to the hepatotoxic effects, the lethal effect of CHCl_3 is also potentiated by exposure to 10 ppm dietary chlordecone (8) (Table 2) and that this is also associated with suppressed repair of the liver tissue (9). Chlordecone also potentiates the hepatotoxicity and lethality of BrCCl_3 (10,11). While the toxicity of these closely related halomethanes is potentiated by such low levels of chlordecone (Figure 2), the toxicity of structurally and mechanistically dissimilar compounds (Figure 3, Table 3) is not potentiated (12) except after exposure to high levels of chlordecone (13). This remarkable capacity to potentiate halomethane hepatotoxicity does not appear to be related to chlordecone-induced cytochrome P450 or associated

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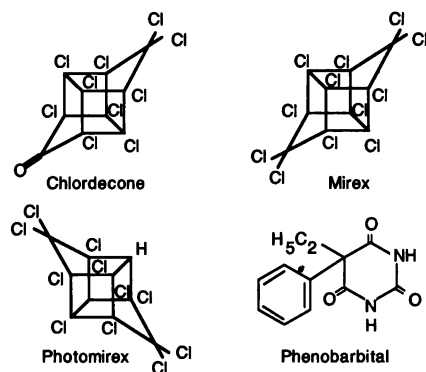
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Table 1. Amplification of lethal effects of several halomethanes by dietary exposure of rats to subtoxic contaminants.

Dietary pretreatment	Halomethane	48 hr LD ⁵⁰ ml/kg	Increase in toxicity-fold
Female rats			
Control	CCl ₄	1.25	—
Chlordecone 10 ppm	CCl ₄	0.048 ^a	26
Male rats			
Control	CCl ₄	2.8	—
Chlordecone 10 ppm	CCl ₄	0.042 ^a	67
Phenobarbital 225 ppm	CCl ₄	1.7	1.6 ^b
Control	BrCCl ₃	0.119	—
Chlordecone 10 ppm	BrCCl ₃	0.027 ^a	4.5

^aHighly significant compared to the respective solvent control. ^bNot significant at $p \leq 0.05$. Mehendale (1); reproduced by permission of *Medical Hypotheses*.

**Figure 1.** Structures of chlordecone, mirex, photomirex, and phenobarbital. Chlordecone (Kepone) amplifies the toxicity of halomethanes closely related to CCl₄. Despite being close structural analogues of chlordecone, mirex and photomirex do not possess this propensity. Phenobarbital, a commonly employed drug in interaction studies at high doses, does increase liver injury of CCl₄, but this enhanced liver injury is inconsequential to animal survival and health, since phenobarbital-treated animals are able to recover from liver injury.**Table 2.** Amplification of lethal effects of halomethanes by dietary exposure of mice to subtoxic contaminants.

Dietary pretreatment	Halomethane	48 hr LD ⁵⁰ ml/kg	Increase in toxicity-fold
Male mice			
Control	CHCl ₃	0.67	—
Chlordecone 10 ppm	CHCl ₃	0.16 ^a	4.2
Mirex 10 ppm	CHCl ₃	0.70	No change
Phenobarbital 225 ppm	CHCl ₃	0.70	No change

^aSignificantly different at $p \leq 0.05$. Mehendale (1); reproduced by permission of *Medical Hypotheses*.

enzymes (2,5,14), enhanced bioactivation of CCl₄ (12,15–18), increased lipid peroxidation (2,12,13), or decreased glutathione (19). Several candidate mechanisms were considered carefully in experiments designed to verify and were found inadequate; additional experiments revealed a novel mechanism (Table 4).

Mechanism of Chlordecone-amplified Toxicity of CCl₄

These findings led to some very basic studies concerning the progression of the hepatotoxicity during a time-course following CCl₄ administration to either normal or chlordecone pretreated rats. The histochemical and histomorphometric experiments revealed that suppressed hepatocellular regeneration and tissue repair (1,2,20,21) might explain the remarkable amplification of CCl₄ toxicity by prior exposure to chlordecone. Similar time-course studies on Ca²⁺ levels in the liver mitochondria, microsomes, and cytosol fractions (22,23) revealed a possible association of increased Ca²⁺ accumulation and suppressed hepatocellular regeneration. Despite some reports that chlordecone interferes with Ca²⁺ uptake mechanisms in extrahepatic tissues (24), chlordecone alone does not cause disruption of hepatocellular Ca²⁺ (25) even at toxic doses. Chlordecone + CCl₄ interaction results in remarkably increased intracellular Ca²⁺ (22–26). Recent studies have also shown a significant activation of phosphorylase α , a finding consistent with the precipitous depletion of glycogen (21,27–29) and ATP (27–29). Based on several lines of experimental evidence, a hypothesis was proposed (1) for the mechanism of the interactive toxicity of chlordecone and CCl₄.

Stimulation of Tissue Repair as a Hormetic Response to Tissue Injury

First, it became necessary to hypothesize the mechanism for why an ordinarily non-

toxic dose of CCl₄ is nontoxic (2). Figure 4 illustrates the mechanism of recovery from limited liver injury observed after the administration of a low dose of CCl₄ alone. Within 6 hr after the administration of a low dose of CCl₄, limited hepatocellular necrosis accompanied by ballooned cells and steatosis inflicted by the same widely accepted mechanisms of CCl₄ bioactivation followed by lipid peroxidation occurs. By mechanisms yet to be understood, simultaneously the liver tissue responds by stimulating hepatocellular regeneration (20,21). Most interestingly, one burst of hepatocellular division is evident at 6 hr, even though centrilobular necrosis only begins to manifest at that time. Although the molecular events responsible for the stimulation of hepatocellular division have not been explored, glycogen, the principal form of hepatic energy resource, is mobilized prior to cell division (20,21). Glycogen levels are restored after cell division has been adequately stimulated (20,21). The limited hepatocellular necrosis enters the progressive phase between 6 and 12 hr (20,21,30,31), while the hepatocellular regeneration and tissue healing processes continue. By 24 hr, no significant liver injury is evident. These observations indicate that stimulation of hepatocellular regeneration is a protective response of the liver, occurs very early after the administration a low dose of CCl₄, and leads to replacement of dead cells, thereby restoring the hepatolobular architecture (2,32,33).

Furthermore, this remarkable biological event results in another important protective action. It is known that newly divided liver cells are relatively resistant to toxic chemicals (34–38). Therefore, in addition to the restoration of the hepatolobular architecture by cell division, by virtue of the relatively greater resistance of the new cells, the liver tissue is able to overcome the imminence of greater injury during the progressive phase (6–12 hr), preventing the spread of injury on the one hand, and

Table 3. Specificity of potentiation of halomethane toxicity by chlordecone.

Compound	Potentiation	Reference
CHCl ₃	yes	Hewitt et al. (6); Purushotham et al. (8)
CCl ₄	yes	Mehendale (2); Curtis et al. (3)
CBrCl ₃	yes	Agarwal and Mehendale (10); Klingensmith and Mehendale (11)
CHBr ₃	no	Mehendale (2)
CBr ₄	no	Mehendale (2)
CCl ₂ CHCl	no	Mehendale and Lockhart (12)
Bromobenzene	no	Mehendale and Lockhart (12)

speeding up the process of overall recovery through tissue healing, on the other (Figure 4). By 6 hr over 75% of the administered CCl_4 is eliminated in the expired air (14) leaving less than 25% in the animal (2). At later time points (12 hr and onwards), most of the CCl_4 will have been eliminated by the animal thereby preventing additional infliction of injury. Continued cellular regeneration during this time period and at later time points allows for complete restoration of the hepatolobular architec-

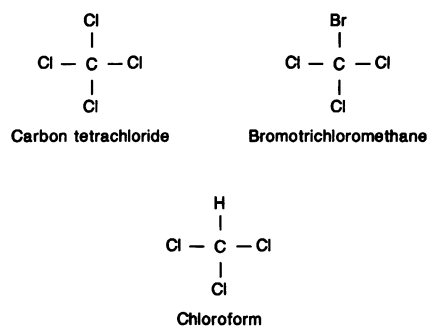


Figure 2. Structures of carbon tetrachloride, bromotrichloromethane, and chloroform as examples of halo-methane solvents. Hepatotoxicity and lethality of these solvents is remarkably amplified by chlordecone.

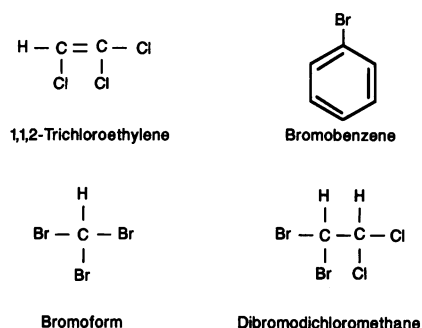


Figure 3. Structure of 1,1,2-trichloroethylene, bromobenzene, bromoform, and dibromodichloromethane. Toxicity of these chemicals is not potentiated by prior dietary exposure to 10 ppm chlordecone.

ture during and after the progressive phase of injury (30,31,39,40). Relative resiliency of the newly divided cells at this critical time frame, as the animal continues to exhale the remaining CCl_4 , is an added critical defense mechanism easily available through cell division.

Administration of the same low dose of CCl_4 to animals maintained on food contaminated with low doses of chlordecone results in initial injury by the same mechanisms of bioactivation of CCl_4 and lipid peroxidation (Figure 4). The liver injury in this case is slightly greater by virtue of approximately doubled rate of bioactivation of CCl_4 in livers of animals pre-exposed to chlordecone (2,14,33). The liver injury thus initiated, enters the progressive phase between 6–12 hr and this phase is accelerated in the absence of tissue repair mechanisms (20,21,30,31,39,40). The highly unusual amplification of CCl_4 toxicity relates to the suppression of the initial hepatocellular regeneration, otherwise ordinarily stimulated by CCl_4 within 6 hr (Figure 4).

The mechanism responsible for the abrogation of this hormetic response of stimulated cell division is of significant interest. Substantial experimental observations indicate that a lack of hepatocellular energy leads to failure of cell division. Under conditions of increased hepatocellular injury, mobilization of hepatic glycogen is initiated in order to stimulate hepatocellular division (21–26). Insufficient energy at a time of increased demand for cellular energy (augmented need for extrusion of extracellular Ca^{2+} from the cells, protection against free-radical mediated injury, and so forth), incapacitates the hepatocytes. As a result, stimulation of cell division, which normally occurs after the administration of a low dose of CCl_4 , cannot occur. The failure of cell division has two important implications: first, hepatolobular structure cannot be restored; second, unavailability of newly divided, relatively resistant cells

predisposes the liver to a permissive continuation of liver injury during the progressive phase (6–12 hr and beyond) (1,2,26,33,41,42). Permissively progressive injury continues unabatedly as a consequence of the mitigated tissue repair mechanisms, leading to massive hepatic failure (1,4,5,10), followed by animal death (1,41–44).

The mechanism underlying a rapid and precipitous decline in cellular ATP is of considerable interest. Many studies have shown a biphasic increase in hepatocellular Ca^{2+} levels in CCl_4 toxicity (23). The unusual aspect of excessive Ca^{2+} accumulation observed in livers treated with the chlordecone + CCl_4 combination is that it occurs at a dose of CCl_4 not ordinarily associated with the causation of increased

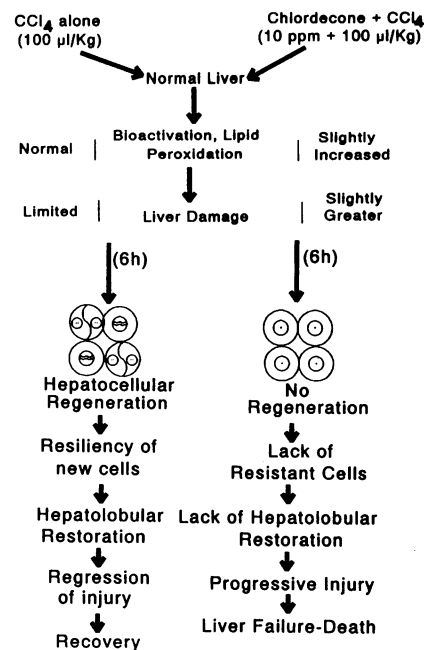


Figure 4. Proposed mechanism for the highly amplified interactive toxicity of chlordecone + CCl_4 . The scheme depicts the concept of suppressed hepatocellular regeneration, simply permitting what is normally limited liver injury caused by a subtoxic dose of CCl_4 to progress in the absence of hepatolobular repair and healing mechanisms stimulated by the limited injury. The limited hepatotoxicity from a low dose of CCl_4 is normally controlled and held in check owing to the hepatocellular regeneration and hepatolobular healing. The chlordecone + CCl_4 combination treatment results in unabated progression of injury owing to lack of tissue repair obtunded due to lack of cellular energy. These events lead to complete hepatic failure, culminating in animal death. Ongoing studies indicate that a very similar mechanism is responsible for the amplification of CHCl_3 and BrCCl_3 toxicity by chlordecone. From Mehendale (1); reproduced with permission of *Medical Hypotheses*.

Table 4. Specificity of potentiation of halomethane toxicity by chlordecone.

Mechanism	Role in amplification
Enhanced bioactivation of halomethanes	Increased infliction of injury Only stage I of toxicity is increased
Increased lipid peroxidation	Not known or none
Estrogenic property of chlordecone	None
Increased Ca^{2+} accumulation	Perturbed cellular biochemistry and ablation of hormetic mechanisms
Precipitous glycogenolysis and loss of ATP	
Suppressed hepatocellular regeneration and unabated progression to stage II of toxicity	Injury becomes irreversible due to ablation of the early-phase hormesis

intracellular Ca^{2+} . Furthermore, chlordecone alone, even at a dose 10-fold higher than used in the interaction studies, does not increase hepatocellular Ca^{2+} (22,25). Although *in vitro* studies with cellular organelles have been employed to speculate that the failure of organelle Ca^{2+} pumps leads to increased cytosolic Ca^{2+} levels, our studies indicate that at no time-point do these organelles contain decreased Ca^{2+} (23,33). Indeed, the only significant change observed with regard to organelle Ca^{2+} is increased Ca^{2+} in the organelles in association with increased liver injury (33,41). Therefore, there is no *in vivo* evidence for decreased Ca^{2+} content in the organelles, which is in contradiction to the predictions from the *in vitro* studies in which organelle incubations were employed to study Ca^{2+} uptake (26,27).

The primary mechanism leading to a highly amplified toxicity is the failure on

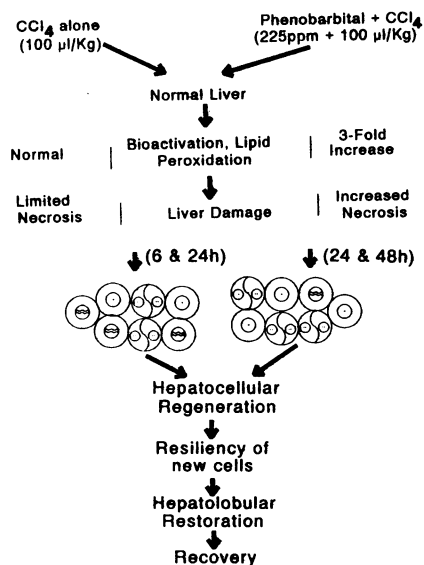


Figure 5. Proposed mechanism for phenobarbital-induced potentiation of CCl_4 -hepatotoxicity in the absence of increased lethality. Normal liver response to a low-dose CCl_4 injury is not abrogated by phenobarbital + CCl_4 interaction. Instead, the early phase of cell division is postponed from the normal 6 to 24 hr. Enhanced putative mechanisms such as increased bioactivation of CCl_4 and resultant increased lipid peroxidation are responsible for the increased infliction of stage 1 injury. Because hepatocellular regeneration and tissue repair processes continue albeit a bit later than normal, these hormetic mechanisms permit tissue restoration resulting in recovery from the enhanced liver injury. This mechanism explains the remarkable recovery from phenobarbital-enhancement of CCl_4 liver injury. Despite a remarkably enhanced liver injury by phenobarbital, this is of no real consequence to the animal's survival because depletion of cellular energy does not occur with this interaction, which permits hormetic mechanisms to restore hepatobular architecture resulting in complete recovery.

the part of the biological events leading to hepatocellular division. Increased accumulation of extracellular Ca^{2+} (23) during the progressive phase of liver injury would be consistent with the significant loss of biochemical homeostasis in hepatocytes (Figure 4). Earlier histomorphometric (21) as well as biochemical studies (28,29,33,41) have shown that glycogen levels drop very rapidly after CCl_4 administration to chlordecone treated animals. Increased cytosolic Ca^{2+} (27) would be expected to result in activation of phosphorylase *b* to phosphorylase *a*, the enzyme responsible for glycogenolysis. Phosphorylase *a* activity (26,27) and precipitous glycogenolysis (20,21,23,27) are observations consistent with the rapid depletion of cellular energy (27) on the one hand, and irreversible increase in cytosolic Ca^{2+} (26) on the other.

An intriguing aspect of the experimental framework leading to the proposed mechanism is the observation that phenobarbital, even at significantly higher doses (225 ppm in the diet for 15 days) does not potentiate the lethal effect of CCl_4 . Although histopathological parameters of liver injury such as hepatocellular necrosis and ballooned cell response are indicative of significantly enhanced hepatotoxicity by phenobarbital, if the animals are left alone, this injury does not progress to significantly increased lethality. Hepatic microsomal cytochrome P450 is approximately doubled by prior dietary exposure to 225 ppm PB and the bioactivation of CCl_4 is tripled (2,14), and these indicators are consistent with the enhanced initiation of liver injury (Stage I of toxicity) measured by histopathology, elevation of serum transaminases, or hepatic function. Nevertheless, the liver injury neither progresses in an accelerated fashion nor is irreversible, as indicated by the reversal of liver injury accompanied by animal survival (2,4,31).

Figure 5 illustrates the proposed mechanism for phenobarbital-enhanced CCl_4 liver injury, which is not associated with increased lethality. Induction of hepatocellular cytochrome P450 results in approximate tripling of CCl_4 bioactivation and increased lipid peroxidation (2,14). Enhanced liver injury is consistent with these observations (Figure 5). It should be recalled that the liver is normally able to respond by stimulation of hepatocellular regeneration after a low dose of CCl_4 within 6 hr (Figure 4). While phenobarbital exposure results in greater injury, the liver's ability to respond by stimulated cell division is not compromised, as evidenced by the stimulation of hepatocellular

regeneration starting at 24 to 36 hr and continuing through 72 hr. Therefore, hepatocellular regeneration is stimulated thereby counteracting the enhanced liver injury, which leads to recovery from increase in liver injury. In view of the enhanced liver injury, restoration of normal hepatobular architecture takes longer than the approximately 24 hr required upon administration of a low dose of CCl_4 alone. Although the hepatocellular regeneration is delayed from 6 to 24 hr, when it does occur it is enhanced substantially, apparently tempered by the demand for more extensive restoration of hepatobular architecture as a consequence of greater injury (8,9,31). Hence, the overall effect of phenobarbital-induced potentiation of CCl_4 injury is merely to delay the stepped up hepatocellular regeneration, tissue repair, and restoration of hepatobular architecture. The prolongation of these normal responses of the liver is a consequence of the enhanced liver injury, inflicted by the enhanced putative injurious mechanisms. Interestingly, hepatic ATP levels were only transiently decreased in phenobarbital pretreated animals upon administration of CCl_4 (27). Availability of cellular ATP at time points beyond 6 hr permits a much stronger response through much higher cell division at 24 hr.

Critical Role of the Early-Phase Stimulation of Cell Division and Tissue Repair

Table 5 presents a variety of experimental manipulations that permit a rigorous experimental verification of the existence and the critical role of tissue repair in the final outcome of toxic injury. The experimental evidence for the existence of a hormetic mechanism was derived as a result of efforts to understand the mechanism of chlordecone potentiation of halomethane toxicity.

Partial Hepatectomy. If the basic premise is valid that suppression of the early-phase (6 hr) stimulation of cell division and tissue repair is the mechanism of chlordecone potentiation of CCl_4 injury, then a preplacement of cell division in the liver should result in protection against the interactive toxicity of chlordecone + CCl_4 . When CCl_4 was administered 2 days after partial hepatectomy at a time of maximally stimulated hepatocellular division, a remarkable protection was observed (43). At 7 days after partial hepatectomy, when the stimulated cell division phases out, the interactive toxicity becomes fully manifested again (43). In these studies, micro-

Table 5. Experimental evidence supporting the proposed mechanism.

Experimental manipulation	Findings	References
1. Preplaced cell division and tissue repair by partial hepatectomy.	Protection from chlordecone + CCl ₄	Kodavanti et al. (30,39,40) Mehendale (41)
2. Toxicity of a large dose of CCl ₄ .	Early-phase stimulation of tissue repair is ablated.	Kodavanti et al. (40); Rao and Mehendale (45)
3. Hepatocytes isolated from chlordecone treated rats incubated with CCl ₄ . (isolated hepatocytes do not divide <i>in vitro</i>)	No potentiation in contrast to <i>in vivo</i> .	Mehendale et al. (46)
4. Developing young rats have growing livers.	Chlordecone does not potentiate toxicity.	Cai and Mehendale (15,16)
5. a. Gerbils lack the early-phase tissue repair.	a. Low dose of CCl ₄ is highly toxic	Cai and Mehendale (15,16)
5. b. Do not have early-phase tissue repair to suppress.	b. Resilient to chlordecone potentiation of CCl ₄ toxicity.	Cai and Mehendale (15,16)
5. c. Preplaced tissue repair by partial hepatectomy.	c. Resiliency to CCl ₄ toxicity.	Cai and Mehendale (48)
6. CCl ₄ autoprotection. phase tissue repair by the protective dose.	Due to prestimulation of early-	Thakore and Mehendale (49)
7. a. Selective ablation of the early-phase hormesis by colchicine. ensues to overcome injury).	a. Prolongation of hepatotoxicity of a low dose of CCl ₄ by 24 hr (until the second phase of cell division at 48 hr	Rao and Mehendale (50,51)
7. b. Colchicine given 2 hr before the protective dose of CCl ₄ .	b. Abolishes CCl ₄ autoprotection entirely.	Rao and Mehendale (52)

From Mehendale (44); reproduced with permission of Lewis Publishers.

somal cytochrome P450 content is decreased by partial hepatectomy, but remains at the decreased level even 7 days later when protection is no longer evident. Moreover, actual *in vivo* bioactivation, and overall disposition of ¹⁴CCl₄ is unperturbed by partial hepatectomy (18).

Large Dose is Toxic Owing to the Ablation of the Hormetic Response. An implication of these findings is that the toxic effect of a large dose of CCl₄ might be a consequence of suppressed early-phase cell division and tissue repair. When a large dose of CCl₄ was administered, the early-phase cell division normally stimulated by a low dose of CCl₄ (20,21,31,40) was ablated entirely (40,45,49). These findings indicate that the real difference between a low and a high dose of CCl₄ is the presence or absence of hormetic response in the

form of stimulated early-phase cell division and tissue repair. The higher dose clearly prevents the hormetic response, thus permissively allowing toxicity to progress unabatedly.

Interactive Toxicity of Chlordecone + CCl₄ Does Not Occur under In Vitro Conditions Where Tissue Hormesis Cannot be Expressed. Yet another line of experimental validation of the critical role of suppressed cell division and tissue repair comes from *in vitro* incubation of hepatocytes isolated from chlordecone pretreated rats with CCl₄ (46). Isolated hepatocytes do not divide under *in vitro* conditions. Therefore, if suppression of cell division and tissue repair ordinarily stimulated by a low dose of CCl₄ is the mechanism of chlordecone-amplified CCl₄ toxicity, one should not observe highly amplified toxic-

ity when hepatocytes from chlordecone treated rats are incubated with CCl₄ *in vitro*. Since prior exposure to phenobarbital is known to result in increased CCl₄ toxicity *in vitro*, incubation of hepatocytes obtained from phenobarbital treated rats with CCl₄ should result in a measurable level of increased toxicity. Such experiments revealed no significant increase in cytotoxic injury in chlordecone-pretreated isolated hepatocyte incubations (46). Cells from phenobarbital pretreated rats exhibited highest CCl₄ toxicity indicating that the *in vitro* paradigm was working as expected. These findings are consistent with the hypothesis that suppression of hepatocellular division and tissue repair is the primary mechanism of chlordecone-potentiated CCl₄ toxicity, and provide substantial evidence against any significant role for chlordecone-enhanced bioactivation of CCl₄ (46).

Resiliency of Newborn and Developing Rats. Newborn and young developing rats have actively growing livers. Since livers during active growth would be expected to have ongoing cell division, these developing rats would be expected to be resilient during their early development. When rat pups at 2, 5, 20, 35, 45, and 60 days were tested, rats were completely resilient to chlordecone potentiation of CCl₄ toxicity up to 35 days of age (38,47). At 45 days, young rats were sensitive to the interactive toxicity of chlordecone + CCl₄ and by 60 days the rats were just as sensitive as adults (47). The hepatic microsomal cytochrome P450 levels in the livers of 35-, 45- and 60-day-old rats exposed to chlordecone were not different from each other suggesting that any differences in cytochrome P450 levels are unlikely to explain the observed differences in toxicities. Moreover, recent studies indicate that bioactivation of ¹⁴CCl₄ in 35-day-old rats is not less than that observed in 60-day-old rats (47). Therefore, the resiliency of younger rats to chlordecone-potentiation of CCl₄ toxicity is more likely related to the ongoing hepatocellular regeneration during early development rather than due to differences in the bioactivation of CCl₄.

Gerbils Lack the Early-Phase Hormesis and Are Most Sensitive to Halomethane Toxicity. While administration of a low dose of CCl₄ to rats results in a prompt stimulation of early-phase hepatocellular regeneration at 6 hr (30,31,39,40,43), in Mongolian gerbils this early-phase cell division is not observed (16). The stimulation of cell division which does occur at 42 hr (analogous to the second

Table 6. High sensitivity of Mongolian gerbils to halomethane toxicity contrasted with their resiliency to potentiation by exposure to other chemicals.

Halomethane	Normal diet	15-Day dietary pretreatment		
		Chlordecone, 10 ppm ^a	Phenobarbital, 225 ppm ^a	Mirex, 10 ppm ^a
CCl ₄ (34–186) ^b	80 (78–128)	100 (28–354)	100 (28–354)	100
CBrCl ₃ (8.6–46.5)	20 (16.4–24.4)	20 (10.4–38.4)	20 (9.9–28.6)	16.8
CHCl ₃ (208–769)	400 (346–923)	565 (268–597)	400 (268–597)	400

^aμl/kg. ^b95% confidence intervals. From Cai and Mehendale (16); reproduced with permission of *Archives of Toxicology*.

phase of cell division which occurs at 48 hr in rats) appears to be too little and too late to be of any help in overcoming liver injury (15,16). If the early-phase cell division is critical for recovery from liver injury, then owing to a lack of this important hormetic mechanism in gerbils, they should be extremely sensitive to halomethane toxicity. When tested, gerbils were found to be approximately 35-fold more sensitive to the toxicity of CCl₄ (15). Likewise, gerbils show several-fold greater sensitivity to the lethal effects of BrCCl₃ and CHCl₃ (Tables 5,6). It follows that gerbils should not be susceptible to chlordecone-potentiation of CCl₄ toxicity (Table 6) since they lack the early phase of hepatocellular regeneration, the target of that interaction (16). Studies have shown that a preplacement of hepatocellular regeneration by partial hepatectomy results in significant protection against CCl₄ toxicity (48), underscoring the importance of stimulated hepatocellular regeneration in determining the final outcome of liver injury. These studies also reveal another important difference between species. While rats respond by maximal stimulation of hepatocellular regeneration within 2 days after partial hepatectomy, in gerbils the maximal stimulation was many-fold lower and it occurs not before 5 days after partial hepatectomy (48). These findings indicate that gerbils are much more sluggish in their hormetic response to a noxious challenge of a hepatotoxic chemical agent. Each of these findings points to the critical importance of the early-phase stimulation of cell division as a decisive target of inhibition in chlordecone-potentiation of CCl₄ toxicity (Table 4). Secondly, these findings also underscore the importance of the biological hormetic response in determining the resiliency to the toxic action of halomethanes.

Autoprotection. CCl₄ autoprotection is a phenomenon, whereby administration of a single low dose of CCl₄ 24 hr prior to the administration of a killing dose of the same compound results in an abolition of the killing effect of the large dose (49–57). The widely accepted mechanism of this phenomenon is the destruction of liver microsomal cytochrome P450 by the protective dose such that subsequently administered large dose is insufficiently bioactivated (32,58–62). Since bioactivation of CCl₄ is an obligatory step for its necrogenic action, it was suggested that massive liver injury ordinarily expected from a large dose of CCl₄ never occurs in the autoprotected animal (32). Although this mechanism has been widely accepted, a closer examination of the evidence suggests that the mechanism was largely derived by association (53–58) rather than actual experimental evidence of less than expected liver injury in the autoprotected animal. Additionally, several lines of evidence indicate that even after the significant destruction of cytochrome P450, the availability of the P450 isozyme responsible for the bioactivation of CCl₄ is not limiting (18,43,47,48,63,64). For instance, even after a 60% decrease in the constitutive liver microsomal cytochrome P450 by CoCl₂ treatment, CCl₄ toxicity was undiminished regardless of whether the rats were pretreated with chlordecone (43). More direct evidence was obtained from studies in which *in vivo* metabolism and bioactivation of ¹⁴CCl₄ were examined in rats pretreated with CoCl₂ (18). The uptake, metabolism, and bioactivation of CCl₄ were not significantly altered in CoCl₂ treated rats known to have highly decreased liver microsomal cytochrome P450 content.

Additional experimental evidence indicating that actual liver injury observed in rats receiving a high dose of CCl₄ was identical regardless of whether prior protective dose was administered led to a reexamination of the mechanism underlying CCl₄ autoprotection (49). A systematic time-course study in which biochemical, histopathological parameters as well as animal survival were examined revealed a critical role for the hormetic response of the liver in the form of stimulated early-phase cell division and tissue repair (49). The protective dose-stimulated tissue repair results in augmented and sustained hepatocellular regeneration and tissue repair, which enable the autoprotected rats to overcome the same level of massive injury, which is ordinarily irreversible and leads to hepatic failure followed by animal death (49,52).

Selective Ablation of the Early-Phase Hormetic Response by Colchicine. Finally, the pivotal importance of the early-phase stimulation of hepatocellular division and tissue repair was tested with an elegant experimental tool, colchicine. With a carefully selected dose of colchicine, it was possible to selectively ablate the early-phase stimulation of mitosis associated with the administration of a low dose of CCl₄ (51,52). One single administration of colchicine at 1 mg/kg results in ablation of mitotic activity, the effect lasting only up to 12 hr, such that the second phase of cell division at 48 hr after the administration of CCl₄ is unperturbed (50). At this dose colchicine does not cause any detectable liver injury nor does it cause any adverse perturbation of hepatobiliary function (51). Therefore use of colchicine permits a very important experimental paradigm in which the early-phase hormesis in response to a low dose of CCl₄ can be selectively ablated. The selective ablation of the early-phase response of cell division resulted in a prolongation of limited liver injury associated with a low dose of CCl₄ (50). Ordinarily, ip administration of 100 μl CCl₄/kg results in very limited liver injury, which is overcome by stimulated cell division and tissue repair (20,21,30,31,39,40,43), within 24 hr. The prolongation of this limited injury lasts only for an additional 24 hr (up to 48 hr after CCl₄ injection) at which time the unperturbed second phase of cell division permits complete recovery to occur within the next 24 hr (by 72 hr after CCl₄ injection). This increased and prolonged CCl₄ injury is not accompanied by enhanced bioactivation of CCl₄ (50,52). Indeed, actual liver injury

assessed by morphometric analysis or hepatocellular necrosis and ballooned cells is not enhanced during the first 12 hr in colchicine treated rats, further indicating that enhancement of the mechanisms responsible for infliction of injury was not responsible (50,52). These findings underscore the pivotal role of the early-phase stimulation of hormesis in the final outcome of toxicity associated with a low dose of CCl_4 .

Another experimental paradigm permits a further test of how critical the early-phase hormetic response is in the final outcome of injury. In the above described experiments, the preservation of the second phase of cell division permits complete recovery by 72 hr. Administration of a large dose of CCl_4 permits one to experimentally interfere with this second phase of cell division. In such an experiment, the animals should not survive because of continued progression of toxicity. In other words, selective ablation of the early-phase hormetic response in an autoprotection protocol should result in a denial of autoprotection. Indeed, 100% survival observed in an experimental protocol (100 μl CCl_4/kg administered 24 hr prior to the injection of 2.5 ml CCl_4/kg) is completely denied by colchicine antimitosis (52). This observation also provides very substantial and convincing experimental evidence for the newly proposed mechanism for the autoprotection phenomenon (49,52). The mechanism underlying the autoprotection phenomenon is the ability of the liver tissue to respond by augmentation of tissue repair through hormesis induced by the protective dose (49).

Two-Stage Model of Toxicity

An intriguing outcome of the work on the interactive toxicity of chlordecone + CCl_4 is the emergence of a concept which permits the separation of the early events responsible for infliction of injury from subsequent events which determine the final outcome of that injury (Figure 6). Hormetic mechanisms (65) are activated upon exposure to low levels of halomethanes (9,20,21,30,31,39,66–68). Although the mechanisms responsible for triggering a dramatic mobilization of biochemical events leading to cellular proliferation within 6 hr after exposure to a subtoxic dose of CCl_4 (9,22,30,31,39) are not understood, it is clear that these early events are the critical determinants of the final outcome of injury (1,33,41,42). When this early phase of hepatocellular division is suppressed, as has been observed in animals pretreated with

chlordecone (20,30,31,39), a permissive and unabated progression of liver injury leading to massive coagulative hepatic necrosis is observed (1,33,41,42). Likewise, experimentally, it has been demonstrated that restoring the tissue hormesis (Figure 7) results in an obtundation of the progressive phase of injury, permitting the tissue to overcome injury.

The central role of hormetic mechanisms in the final outcome of tissue injury becomes self-evident from the following lines of experimental evidence. Prior exposure to 225 ppm phenobarbital results in the potentiation of liver injury by the same subtoxic dose of CCl_4 employed in the

chlordecone + CCl_4 interaction (1,2,4,31). The quantitative measures of liver injury at 24 hr after the administration of CCl_4 indicate that the tissue injury is either equivalent to or slightly greater than that seen in chlordecone + CCl_4 interaction (2). Left alone, the animals undergoing the toxicity of phenobarbital + CCl_4 combination recover, while those experiencing the chlordecone + CCl_4 combination do not (1,4,33,41,42). While the enhanced liver injury observed with the toxicity of phenobarbital + CCl_4 is consistent with the increased bioactivation of CCl_4 (2,14), recovery from this injury is consistent with the unabated hepatocellular proliferation and tissue repair (31,39). Delayed hepato-

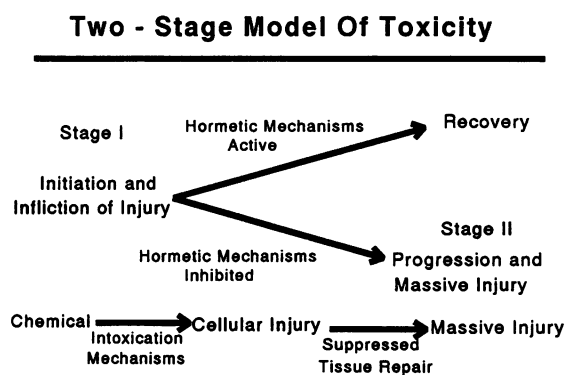


Figure 6. Scheme illustrating the proposed two-stage model of toxicity. Stage I involves infliction of cellular and/or tissue injury by intoxication mechanisms, which are understood for many chemical and physical agents. When injury is inflicted by a low dose of the offending agent (stage I), hormetic mechanisms are stimulated (such as cellular regeneration and tissue repair targeted for restoration of tissue structure) and complete recovery from injury follows with no additional toxic consequence. If hormetic mechanisms are suppressed or ablated, the limited injury associated with exposure to a low dose of the offending toxic agent would continue unabated resulting in progressive injury. High doses of toxic agents can cause ablation of the hormetic mechanism, as in the case of high dose of CCl_4 , which results in ablation of the early-phase hormetic response (40). Another example is the ablation of the early-phase hormesis exemplified by the interactive toxicity of chlordecone and the halomethane solvents. From Mehendale (42); reproduced with permission of Lewis Publishers.

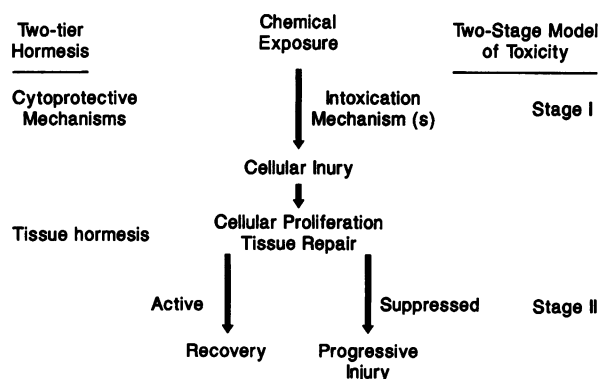


Figure 7. Scheme illustrating the concept of separating those mechanisms which are responsible for the infliction of cellular and tissue injury from those which come to follow these events. Intoxication mechanisms result in infliction of injury during stage I of toxicity. During this stage tissue hormetic mechanisms are stimulated in an attempt to overcome injury. If these hormetic mechanisms are unperturbed, recovery occurs. Interference with these mechanisms results in uncontrollable progression of injury, resulting in stage II of toxicity.

cellular regeneration and tissue repair from the normal 6 hr to 24 to 36 hr (1,31) is the only consequence on stage II of CCl_4 toxicity. Nevertheless, the highly stimulated early phase of tissue repair at 24 hr enables the restoration of hepatobular structure and function (1,33,41,42,44), and thereby animal survival. These observations provide additional support for the concept of two distinct stages of chemical toxicity (Figure 7).

Induction of liver regeneration 36 to 48 hr after the administration of a toxic dose of CCl_4 is well established (69–71). The existence of an early phase of cell division (6 hr) was revealed only through experiments with a low, subtoxic dose of CCl_4 (20,21,30,31). In fact, administration of a large, toxic dose of CCl_4 (2.5 ml/kg) results in complete suppression of this early phase of cell division (40,45,49), indicating that the toxicity associated with a large dose is due to the abolishment of this critical early phase stimulation of tissue repair (1,33,41,42). Therefore, it is possible to ablate the early phase of hepatocellular regeneration and tissue repair ordinarily stimulated by a low dose of CCl_4 , making it in essence a toxic dose. Administration of the same dose to animals prestimulated by partial hepatectomy so that they have the ongoing hepatocellular proliferation and tissue repair, results in a remarkable and substantial protection from liver injury and lethality (45). Likewise, administration of a large lethal dose of CCl_4 to animals receiving a smaller dose to stimulate cell division and tissue repair results in complete protection (49,52). Such protection is not due to decreased bioactivation of CCl_4 (18,50).

The importance of the stimulation of tissue repair as an event independent of stage I of chemical toxicity can be illustrated by other elegant experimental approaches. Experimental interference with the early phase of hepatocellular proliferation leads to prolonged and enhanced liver injury of an ordinarily subtoxic dose of CCl_4 . Studies with colchicine antimetabolism (50–52), wherein colchicine dose administered selectively ablates the early phase of hepatocellular division (6 hr) without interfering with the second phase of hepatocellular regeneration (48 hr), have shown a prolongation of liver injury. Neither liver injury measured through serum enzyme elevations nor that measured by morphometric analysis of necrosis was increased at 6 or 12 hr in colchicine treated rats, findings consistent with the lack of colchicine-enhanced bioactivation of CCl_4 (50,52). Moreover, colchicine ablation of the early-phase hormetic response after the protec-

tive dose of CCl_4 in an autoprotection protocol leads to complete denial of autoprotection.

The critical role played by the capacity to respond to CCl_4 -hepatotoxicity by stimulation of tissue repair mechanisms at an early time point is illustrated by examining species and strain differences in susceptibility to CCl_4 injury. Mongolian gerbils are extremely sensitive to halomethane hepatotoxicity (15,16,48,72). Gerbils are approximately 35-fold more sensitive to CCl_4 toxicity than Sprague-Dawley rats (15,16). This difference in CCl_4 toxicity can be seemingly explained on the basis of a 3.5-fold greater bioactivation of CCl_4 in gerbils (15). However, the remarkable and substantial sensitivity does not appear to be due to 3.5-fold greater bioactivation of CCl_4 , since CCl_4 toxicity is not at all increased in gerbils by prior exposure to phenobarbital in spite of a 5-fold greater bioactivation of CCl_4 (15,16). The time-course studies on the ability of gerbils to respond to a subtoxic dose of CCl_4 by stimulation of hepatocellular regeneration and tissue repair reveal an important difference in the biology of the hormetic mechanisms between gerbils and rats (16). The early-phase stimulation of tissue repair in the liver does not manifest itself in gerbils and the second phase occurs approximately 40 hr after the administration of CCl_4 (16,48). In the absence of the biological mechanism to arrest the progression of liver injury (Figure 7), the liver injury might be expected to permissively progress much like an unquenched brushfire.

Evidence in support of the concept that species differences in chemical toxicity might depend on the differences in the promptness in initiating tissue repair mechanisms among various species comes from another aspect of the interactive toxicity of chlordecone + CCl_4 . While gerbils are extremely sensitive to CCl_4 , this sensitivity cannot be further increased by prior exposure to chlordecone (15,16,48,72). Since substantial evidence supports the concept that suppression of the early phase of hepatocellular regeneration and tissue repair is the mechanism for the permissive progression of liver injury in the chlordecone + CCl_4 interaction (1,33,41,42,44), lack of this early phase response in the gerbil would be consistent with extremely high sensitivity of gerbils to CCl_4 on the one hand, and a lack of potentiation of CCl_4 toxicity by prior exposure to chlordecone on the other (15,16). This concept has received additional support through partial hepatectomy experiments (48).

The toxicity of chlordecone + CHCl_3 combination has been demonstrated in murine species (6–9). Stimulation of hepatocellular regeneration and tissue repair after a subtoxic dose of CHCl_3 allows the mice to overcome the liver injury associated with that dose of CHCl_3 (9). By lowering the dose of CHCl_3 used in the chlordecone + CHCl_3 studies (8), it is possible to demonstrate potentiation of liver injury, but without the lethality (9). Such an experimental protocol vividly reveals a decisive role played by the stimulated tissue repair mechanisms in overcoming liver injury (9) and the separation of these mechanisms (stage II) from the infictive phase (stage I) of chemical injury (Figure 7).

The importance of stimulated tissue repair mechanisms in overcoming liver injury has also been demonstrated through examination of the mechanistic basis for significant strain differences in mice (73,74). An SJL/J strain of mice, known to be least susceptible to CCl_4 toxicity, was shown to possess more prompt and efficient tissue repair mechanisms, which permit augmented recovery, while the BALB/C strain, known to be more susceptible, was shown to possess less efficient tissue repair mechanisms resulting in slow recovery (73). The F_1 cross between these two strains was shown to be intermediate in susceptibility (74). A careful histopathological evaluation revealed that while the time course of the appearance of injury was quite similar (stage I, Figure 6), significant differences in tissue repair mechanisms between these strains could account for the strain differences in CCl_4 toxicity (73,74). While the time course of the infictive phase of injury in the F_1 (SJL/J \times BALB/C) was similar to the two parent strains, the tissue repair was at the intermediate level of augmented (SJL/J) and retarded (BALB/C) recovery.

With the advent of the finding that a low dose of CCl_4 is not toxic, not so much because it does not initiate tissue injury, but because of the stimulated tissue repair mechanisms (44), it became apparent that the stimulation of the early phase of hepatocellular regeneration is in essence an endogenous hormetic mechanism, recruited to overcome tissue injury. One implication of this finding is its possible role in the phenomenon of CCl_4 autoprotection (53–55,60). Circumstantial evidence, wherein hepatic microsomal cytochrome P450 was decreased by CoCl_2 administration to 40% of the normal level did not result in decreased CCl_4 liver injury (43), suggested the possibility that

mechanism(s) other than decreased cytochrome P450 might be involved in CCl_4 autoprotection. Recent studies reveal a critical role for the hepatocellular regeneration and tissue repair stimulated by the low protective dose administration (49). Essentially, the protective dose serves to stimulate tissue repair mechanisms (18,20,21,30,39) so that even before the large dose known to abolish the early phase stimulation of tissue repair (40) is administered, the tissue repair mechanisms are already in place, resulting in augmentation of tissue repair sufficient to tip the balance between injury and recovery in favor of the latter (49). This experimental model represents another example wherein a selective augmentation of the tissue hormetic mechanism (stage II, Figure 6) independent of the inflicative phase of toxicity (stage I, Figure 6), one can dramatically alter the ultimate outcome of toxic injury (Figure 7).

Another line of evidence to implicate the importance of the hormetic mechanisms in determining the final outcome of chemical toxicity comes from experiments designed to understand the mechanisms responsible for the failure of the tissue regenerative and repair mechanism in the interactive toxicity of chlordecone + CCl_4 . Much evidence is available to implicate insufficient availability of cellular energy at a time when cell division should have taken place (20,21,75). A remarkable and irreversibly precipitous decline in glycogen levels in the liver (21,26), a rise in hepatocellular Ca^{2+} (22–25), a consequent stimulation of phosphorylase α activity, leading to an equally precipitous decline in hepatic ATP (26,27), are events consistent with the failure of hepatocellular regeneration in the chlordecone + CCl_4 interaction. Only marginal and transient decline in ATP levels in the interactive hepatotoxicity of phenobarbital + CCl_4 and mirex + CCl_4 (28) are consistent with only a postponement of hepatocellular regeneration leading to transiently increased liver injury followed by complete recovery (31). The concept of insufficient hepatocellular energy being linked to failure of hepatocellular regeneration and tissue repair has gained support from experiments in which the administration of external source of energy resulted in augmented ATP levels and significant protection (28,29,45). Catechin (cyanidanol), known to increase hepatic ATP levels, protects against the lethal effect of chlordecone + CCl_4 (28,29). Protection by catechin is accompanied by a restored stimulation of hepatobular repair and tissue healing (29). The most interesting

Table 7. Chemicals reported to cause nonneoplastic hepatocellular proliferation.

Chemicals	References
1. Acetaminophen	Zieve et al. (76)
2. Allyl alcohol	Zieve et al. (76,77)
3. α -Naphthyl isothiocyanate	McClellan and Rees (78) Ungar et al. (79)
4. Bromotrichloromethane	Faroon and Mehendale (66)
5. Carbon tetrachloride	Lockhart et al. (20,21) Nakata et al. (71)
6. Chloroform	Condie et al. (80)
7. Ethylene dibromide	Natchomie and Farber (81)
8. Galactosamine	Lesch et al. (82) Kuhlmann and Wurster (83)
9. Thioacetamide	Gupta (84) Reddy et al. (86)

aspect of catechin protection against the interactive toxicity of chlordecone + CCl_4 is that protection does not appear to be the result of decreased infliction of hepatic injury (28,29), as evidenced by a lack of difference in injury up to 24 hr after CCl_4 administration (29). These observations provide substantial evidence for the separation of stage I of toxicity responsible for the infliction of tissue injury from the stage II events responsible for the final outcome of tissue injury (42).

Abundant opportunities are available to test the two-stage of model toxicity. Many chemicals have been reported to induce hepatocellular regeneration at relatively modest doses, some of which are listed in Table 7. Opportunities to test the conceptual framework being put forth here are available through additional investigations with these models of tissue injury as well as scores of other models in other tissues and organs.

Implications for Assessment of Risk to Public Health

Establishing that the initial toxic or injurious events, regardless of how they are caused, can be separated from the subsequent events that determine the ultimate outcome of injury, offers promising opportunities for developing new avenues for therapeutic intervention with the aim of restoring the hormetic tissue repair mechanisms. Such a development will open up avenues for two types of measures to pro-

tect public health. The presently used principle is to decrease injury by interfering with stage I of toxicity by treatment with an antidote, which either prevents further injury or decreases already inflicted injury. The second, wherein tissue repair and healing mechanisms could be enhanced not only to obtund the progression of injury, but also to simultaneously augment recovery from that injury, is a novel approach.

In addition to these opportunities, the two-stage concept of chemical toxicity also embodies implications of significant interest in the assessment of risk from exposure to toxic chemicals. The existence of a threshold for chemical toxicity is evident as indicated by the stimulation of tissue repair mechanism directed to tissue healing and recovery observed after the administration of subtoxic levels of toxic chemicals, when exposure involves singular chemicals. The existence of a two-level or two-stage threshold is apparent from the two-tier hormetic response: one threshold for each stage of the two-stage model. Generally speaking, the threshold for stage I of toxicity must lie in the cytoprotective mechanisms (cellular hormesis). The threshold for stage II of toxicity appears to be in the tissue's ability to respond promptly by augmenting tissue healing mechanisms. These thresholds may be quantitatively the same or different.

From a public health perspective, exposure to singular chemicals is seldom involved. Multiple exposures to chemical combinations and solidus or singular components simultaneously, intermittently, or sequentially are almost always the rule. In this regard, antagonistic interactive toxicity or inconsequential interactions are also of interest. Of greater interest from a public health perspective, is the finding that the hormetic mechanisms which constitute the threshold for physical or chemical toxicity can be mitigated by other chemical and physical agents, resulting in highly accentuated toxicity.

Of significantly greater interest is the need to take into account the hormetic mechanisms operating particularly at the low levels of exposure to chemicals, in the assessment of risk from exposures to combinations of chemicals at low doses. The recognition of the existence of cellular and tissue hormesis provides a mechanistic basis to recognize thresholds for toxic effects, thereby permitting us to take into consideration the lack of recognizable adverse health effects at low levels of exposure to chemicals in our environment.

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